

Application No.: 09/840,125  
Amendment Dated 3 February 2004  
Reply to Office Action of 3 October 2003

*AMENDMENTS TO THE CLAIMS*

This Listing of Claims will replace all prior versions, including listings, of claims in the application.

*Listing of Claims*

Claims 1-25 (canceled).

Claim 26 (currently amended): An isolated DNA comprising a sequence of SEQ ID NO:3 as altered by one or more mutations selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, ~~G5349A~~ and G5360A.

Claim 27 (currently amended): A nucleic acid probe specifically hybridizable to a human mutated *SCN5A* and not to wild-type *SCN5A* DNA, said mutated *SCN5A* comprising a mutation of SEQ ID NO:3 selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, ~~G5349A~~ and G5360A.

Claim 28 (currently amended): A method for detecting a mutation in *SCN5A* said mutation selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, ~~G5349A~~ and G5360A which comprises analyzing a sequence of said gene or RNA from a human sample or analyzing the sequence of cDNA made from mRNA from said sample.

Claim 29 (original): The method of claim 28 wherein said mutation is detected by a method selected from the group consisting of:

a) hybridizing a probe specific for one of said mutations to RNA isolated from said human sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

- b) hybridizing a probe specific for one of said mutations to cDNA made from RNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;
- c) hybridizing a probe specific for one of said mutations to genomic DNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;
- d) amplifying all or part of said gene in said sample using a set of primers to produce amplified nucleic acids and sequencing the amplified nucleic acids;
- e) amplifying part of said gene in said sample using a primer specific for one of said mutations and detecting the presence of an amplified product, wherein the presence of said product indicates the presence of said mutation in the sample;
- f) molecularly cloning all or part of said gene in said sample to produce a cloned nucleic acid and sequencing the cloned nucleic acid;
- g) amplifying said gene to produce amplified nucleic acids, hybridizing the amplified nucleic acids to a DNA probe specific for one of said mutations and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation;
- h) forming single-stranded DNA from a gene fragment of said gene from said human sample and single-stranded DNA from a corresponding fragment of a wild-type gene, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to wild-type and sequencing said single-stranded DNA having a shift in mobility;
- i) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding human wild-type gene fragment,

analyzing for the presence of a mismatch in said heteroduplex, and sequencing said first strand of nucleic acid having a mismatch;

j) forming single-stranded DNA from said gene of said human sample and from a corresponding fragment of an allele specific for one of said mutations, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to said allele, wherein no shift in electrophoretic mobility of the single-stranded DNA relative to the allele indicates the presence of said mutation in said sample; and

k) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment of said gene isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding gene allele fragment specific for one of said mutations and analyzing for the presence of a mismatch in said heteroduplex, wherein no mismatch indicates the presence of said mutation.

Claim 30 (original): A method according to claim 29 wherein hybridization is performed *in situ*.

Claims 31-33 (canceled).

Claim 34 (currently amended): A method of assessing a risk in a human subject for long QT syndrome which comprises screening said subject for a mutation in *SCN5A* selected from D1114N, L1501V, delF1617, R1623L, and S1787N by comparing the sequence of said *SCN5A* or its expression products isolated from a tissue sample of said subject with a wild-type sequence of said *SCN5A* or its expression products, wherein a mutation selected from D1114N, L1501V, delF1617, R1623L, and S1787N in the sequence of the subject indicates a risk for long QT syndrome.

Claim 35 (original): The method of claim 34 wherein said expression product is selected from mRNA of said gene or a polypeptide encoded by said gene.

Claim 36 (original): The method of claim 34 wherein one or more of the following procedures is carried out:

- (a) observing shifts in electrophoretic mobility of single-stranded DNA from said sample on non-denaturing polyacrylamide gels;
- (b) hybridizing a probe to genomic DNA isolated from said sample under conditions suitable for hybridization of said probe to said gene;
- (c) determining hybridization of an allele-specific probe to genomic DNA from said sample;
- (d) amplifying all or part of said gene from said sample to produce an amplified sequence and sequencing the amplified sequence;
- (e) determining by nucleic acid amplification the presence of a specific mutant allele in said sample;
- (f) molecularly cloning all or part of said gene from said sample to produce a cloned sequence and sequencing the cloned sequence;
- (g) determining whether there is a mismatch between molecules (1) said gene genomic DNA or mRNA isolated from said sample, and (2) a nucleic acid probe complementary to the human wild-type gene DNA, when molecules (1) and (2) are hybridized to each other to form a duplex;
- (h) amplification of said gene sequences in said sample and hybridization of the amplified sequences to nucleic acid probes which comprise wild-type gene sequences;
- (i) amplification of said gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise said mutant gene sequences;
- (j) screening for a deletion mutation;
- (k) screening for a point mutation;
- (l) screening for an insertion mutation

- (m) determining *in situ* hybridization of said gene in said sample with one or more nucleic acid probes which comprise said gene sequence or a mutant sequence of said gene;
- (n) immunoblotting;
- (o) immunocytochemistry;
- (p) assaying for binding interactions between said gene protein isolated from said tissue and a binding partner capable of specifically binding the polypeptide expression product of a mutant allele and/or a binding partner for the polypeptide; and
- (q) assaying for the inhibition of biochemical activity of said binding partner.

Claim 37 (currently amended): A nucleic acid probe which hybridizes to the isolated DNA of claim 26 under conditions at which it will not hybridize to wild-type SCN5A DNA.

Claim 38 (original): A method for diagnosing a mutation which causes long QT syndrome comprising hybridizing a probe of claim 37 to a patient's sample of DNA or RNA, the presence of a hybridization signal being indicative of long QT syndrome.

Claim 39 (original): A method according to claim 38 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized with a probe of claim 37.

Claim 40 (original): A method according to claim 38 wherein said hybridization is performed *in situ*.

Claim 41 (original): A method according to claim 38 wherein said assay is performed using nucleic acid microchip technology.

Claim 42 (currently amended): A method for diagnosing a mutation which causes long QT syndrome comprising amplifying a region of gene or RNA for SCN5A and sequencing the amplified gene or RNA wherein long QT syndrome is indicated by any one or more mutations

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selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, ~~G5349A~~ and G5360A.

Claim 43 (currently amended): A method for diagnosing a mutation which causes long QT syndrome comprising identifying a mismatch between a patient's DNA or RNA and a wild-type DNA or RNA probe wherein said probe hybridizes to a region of DNA or RNA wherein said region comprises a mutation of SEQ ID NO:3 selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, ~~G5349A~~ and G5360A.

Claim 44 (original): The method of claim 43 wherein the mismatch is identified by an RNase assay.

Claims 45-49 (canceled).

Claim 50 (currently amended): An isolated DNA encoding an SCN5A polypeptide of SEQ ID NO:4 having a mutation selected from the group consisting of D1114N, L1501V, delF1617, R1623L, ~~E1784K~~ and S1787N.